Inaccuracy of Calculated LDL-Cholesterol in Type 2 Diabetes: Consequences for Patient Risk Classification and Therapeutic Decisions, Ana María Wagner, José Luis Sánchez-Quesada, Antonio Pérez, Mercedes Rigla, Mariano Carbó, Francisco Blanco-Vaca, and Jordi Ordóñez-Llanos (Departments of 1 Endocrinology and Nutrition and 2 Biochemistry, and 3 Research Institute, Hospital de Sant Pau, 08025 Barcelona, Spain; 4 Department of Biochemistry and Molecular Biology, Universitat Autònoma, 08025 Barcelona, Spain; * address correspondence to this author at: Department of Biochemistry, Hospital de Sant Pau, Avgda Sant Antoni Mª Claret 167, 08025 Barcelona, Spain; fax 34-93-2919196, e-mail 8@hsp.santpau.es)

LDL-cholesterol (LDLc) is the main lipid marker in cardiovascular risk estimation and the principal therapeutic target in both diabetic and nondiabetic subjects (1, 2). The designated comparison method for the determination of LDLc, using ultracentrifugation and precipitation, known as “β-quantification” (3), is cumbersome and time-consuming and requires expensive instrumentation and trained personnel. The Friedewald equation (4) (LDLc = total cholesterol – HDLc – [triglycerides (in mmol/L)/2.17 or triglycerides (in mg/dL)/5]), the most frequently used method for the calculation of LDLc, assumes that VLDL particles maintain a nearly constant cholesterol: triglyceride ratio. However, this assumption is invalid in the presence of chylomicronemia and increased VLDL or intermediate-density lipoprotein particles (4–7).

Because diabetic dyslipidemia includes quantitative and qualitative abnormalities in lipoprotein particles, including VLDL and their remnants (8–10), the use of the Friedewald equation in diabetic patients has been questioned (11–13). HDL-cholesterol (HDLc), often determined after chemical precipitation of apolipoprotein B (apoB)-containing lipoproteins, has technical drawbacks that could interfere with the accuracy of LDLc calculation (14). New homogeneous, direct methods have improved HDLc determination (15). However, the consequences on patient classification and therapy of using direct, more precise methods for HDLc in the estimation of LDLc by the Friedewald equation have, to our knowledge, not been assessed.

We previously proposed an equation that included total triglycerides and cholesterol, and apoB that was more accurate than the Friedewald equation in estimating LDLc (16). Because diabetic dyslipidemia includes hyperapoB (17), an equation that includes apoB in the estimation of LDLc could be of special interest in these patients. Thus, our aims were to ascertain whether a direct HDLc method increases the accuracy of the Friedewald formula, to evaluate an equation that includes apoB in the estimation of LDLc, and to assess the proportion of patients misclassified by the different equations and the therapeutic consequences of that misclassification in type 2 diabetic patients. Comparisons were made against β-quantification.

Ninety-five consecutive nonchylomicronemic type 2 diabetic patients (61% male; age, 57.7 ± 10.7 years, mean ± SD), with a mean diabetes duration of 10 years (range, 0–33 years) and mean glycohemoglobin of 7.9% (5.7–14%) were studied; 58% received insulin therapy, 54% had microangiopathy, and 31% had macroangiopathy. Dysbetalipoproteinemia was ruled out by a VLDL-cholesterol/total triglyceride ratio >0.65. Dyslipidemia was defined using the following cutoff points: 2.25 mmol/L for total triglycerides and 4.13 mmol/L for LDLc (2). HyperapoB was defined according to a previously obtained cutoff point of 1.1 g/L (17). Blood samples from 183 nondiabetic subjects were consecutively selected, after excluding those on lipid-lowering or any other drugs or situations known to affect lipoprotein metabolism. All subjects gave written informed consent.

Patients were stratified according to the LDLc concentrations obtained by the different methods, following the cutoff points recommended by the National Cholesterol Education Program (NCEP) to define risk categories: ≤2.59, 2.60–3.36, 3.37–4.13, 4.14–4.91, and >4.91 mmol/L. They were also classified according to the LDLc concentration above which pharmacological intervention is recommended (3.36 mmol/L for patients without and 2.59 mmol/L for patients with previous cardiovascular events) (1).

Total cholesterol and triglycerides were measured by enzymatic methods (18, 19) (CHOP-PAP and GPO-PAP, respectively; Roche Diagnostics). Total cholesterol was calibrated using calibration material from Roche, with a value assigned by the modified Abell-Kendall method recommended by the CDC. HDLc was measured by both precipitation, using phosphotungstate/MgCl2, and by a direct method (both from Roche Diagnostics) (20). External quality-control programs rendered mean inaccuracies and imprecisions lower than ±2.6% and 2.0%, respectively, for all of the methods described above. apoB was measured by immunoturbidimetry (Roche Diagnostics), standardized against WHO/IFCC SP3-07 (21). Between-batch imprecisions and inaccuracies of the apoB assay, assessed by commercial controls (Precinorm-L and Precipath-L; Roche Diagnostics) were 4.4% and 2.6% and -0.6% and -2.6%, respectively.

LDLc was calculated by the Friedewald formula using the HDLc value obtained by precipitation (LDLc-Fp) and by the direct method (LDLc-Fd), and was also determined by a modified β-quantification method (LDLc-R) separating VLDL at <1.006 kg/L by ultracentrifugation (at 105,000 g for 18 h at 4 °C) and measuring HDLc after precipitation in the infranatant with phosphotungstate/MgCl2 (2).

A multiple regression analysis was performed to identify the best predictors of LDLc-R in the control population, and an equation that includes apoB (in g/L), triglycerides, and total cholesterol (both in mmol/L) was obtained [LDLc-apoB = (0.385 × total cholesterol) + (2.010 × apoB) – (0.342 × triglycerides); r = 0.994; P <0.001] and used to calculate LDLc in the diabetic population. Bias against LDLc-R was evaluated for all three equations at ± 4%, as recommended by the NCEP (5), and at ± 10%, which is used frequently in clinical settings.
Table 1. Bias of LDLc estimation by the three equations in all type 2 diabetic patients, compared with the recommended method, and therapeutic approach derived from their application.

<table>
<thead>
<tr>
<th>Percentage of results with bias</th>
<th>Therapeutic approach</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correct approach, % (kappa index)</td>
</tr>
<tr>
<td>Less than ±4%</td>
<td>Greater than −4%</td>
</tr>
<tr>
<td>LDLc-Fp</td>
<td>27.4</td>
</tr>
<tr>
<td>LDLc-Fd</td>
<td>31.6</td>
</tr>
<tr>
<td>LDLc-apoB</td>
<td>46.3</td>
</tr>
</tbody>
</table>

* tmt, treatment.

SPSS 8.0 for Windows (SPSS Inc) was used for statistical analysis. Differences between groups were analyzed by the Student or Mann–Whitney tests, and a paired t-test was used to compare means within a group. \( P < 0.05 \) was considered significant. Concordance between the different equations and LDLc-R in the diagnosis and treatment of patients was assessed by kappa (k) index (0.21–0.40, 0.41–0.60, 0.61–0.80, and 0.81–1.0, which show fair, moderate, good, and very good concordance, respectively) (22).

Ninety-one of 95 patients had triglyceride concentrations <4.6 mmol/L, and apoB was increased in 26 of the 27 hypercholesterolemic and in 35 of the 68 normocholesterolemic subjects. The results obtained by LDLc-apoB (3.78 ± 0.77 mmol/L) were equivalent to those obtained by LDLc-R (3.76 ± 0.86 mmol/L), whereas results obtained by LDLc-Fp (3.49 ± 0.84 mmol/L) and LDLc-Fd (3.52 ± 0.84 mmol/L) were lower (\( P < 0.005 \); see Table 1).

Table 1 compares the bias of the equations, and Fig. 1 shows the accuracy of patient classification into risk categories according to LDLc estimation by the different equations compared with LDLc-R. The best concordance was obtained by LDLc-apoB, the only equation to achieve a k >0.6. According to their LDLc-R concentrations, 44 of the 66 patients who had not and 27 of the 29 who had suffered a cardiovascular event were candidates for drug therapy. Table 1 shows the correct and incorrect therapeutic decisions that resulted from the application of international guidelines (1) to the different LDLc estimations.

Cardiovascular disease is highly prevalent and is the principal cause of death in diabetic subjects (23, 24). This high risk can to a certain extent be explained by the lipid abnormalities found in this population (10). LDLc, albeit often normal or only slightly increased in type 2 diabetic patients, is the main marker used to assess cardiovascular risk and make therapeutic decisions (1, 2). To our knowledge, this is the first time that the impact of the Friedewald equation on therapeutic approach has been evaluated after individual patient assessment. The impact of LDLc estimation on patient classification has previously been evaluated using NCEP risk categories alone, without taking patients’ cardiovascular disease histories into account. However, we have tried to stress the importance of taking patients, not just their numbers, into consideration. Our results show that a new equation that includes apoB allows a more accurate estimation of LDLc than the Friedewald equation, with consequences on patient risk assessment and treatment.

In agreement with most of the studies performed on type 2 diabetic subjects (12, 13), the LDLc concentrations obtained by both forms of the Friedewald equation were significantly lower than LDLc-R. Furthermore, the present results suggest that direct HDLc measurements not only are equivalent to those based on precipitation (25), as recommended by the NCEP (14), but can also somewhat improve LDLc calculation (Table 1). LDLc-apoB achieved a lower bias; its mean value was indistinguishable from LDLc-R. Most patients had triglyceride concentrations <4.6 mmol/L, which means that the advantage of the new formula is not attributable to inappropriate application of the Friedewald equation. Diabetic dyslipidemia includes lipoprotein abnormalities, which may cause underestimation of LDLc by the Friedewald formula. On the other hand, LDL particles contain >90% of total apoB, and each LDL particle carries one apoB molecule (26). Thus, a good estimation of LDLc should be expected when total triglycerides, total cholesterol, and apoB are used for its calculation.

Albeit not inexpensive, the apoB assay also adds important clinical information for the evaluation of cardiovascular risk because increased apoB not only is associated with cardiovascular disease (27, 28), but frequently is found in normocholesterolemic type 2 diabetic patients (17). The classification of patients into risk categories is
used to design therapeutic strategies. Almost 75% of the patients we assessed were eligible for pharmacological treatment. Thus, the need for an accurate estimation of LDLc to determine therapeutic intervention is evident. Nevertheless, unlike in the present study, this point has, to our knowledge, previously been assessed based on lipid concentrations alone. Both forms of the Friedewald equations underestimated cardiovascular risk and the need for drug intervention, which would be omitted inappropriately in ~10% vs in no cases according to LDL-apoB.

In conclusion, equations used to calculate LDLc concentrations in type 2 diabetes are far from ideal. The inclusion of apoB in the estimation decreases its bias and allows identification of additional patients at risk. Until direct LDLc methods have been thoroughly assessed, we may recommend that the proposed formula be used for LDLc estimation in type 2 diabetic patients.

References

Presence of Fetal RNA in Maternal Plasma, Leo L.M., Poont, Tse N. Leung, Tze K. Lau, and Y.M. Dennis Lo*
(Departments of 1Chemical Pathology and 2Obstetrics and Gynecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR; * address correspondence to this author at: Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Room 38023, 1/F Clinical Sciences Building, 30-32 Ngan Shing Street, Shatin, New Territories, Hong Kong SAR; fax 852-2194-6171, e-mail loym@cuhk.edu.hk)

The discovery of fetal DNA in maternal plasma (1) has opened up a new horizon on prenatal molecular diagnosis. Many groups have since shown that fetal genetic traits, such as RhD status and inherited genetic diseases, can be determined from fetal DNA in maternal plasma (2–5). However, it is not known whether fetal RNA is also present in maternal plasma. Here, using a two-step reverse transcription (RT)-PCR assay, we demonstrate the presence of fetal-derived, male-specific mRNA in plasma of pregnant women carrying male fetuses.

Pregnant women attending the Prenatal Diagnosis Unit at the Department of Obstetrics and Gynecology, Prince of Wales Hospital, Hong Kong were recruited with informed